IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
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ETSUO OSHIMA, et al.	)	
	:	Group Art Unit: 129
Serial No.: 020,900	)	
	:	Examiner:
Filed: March 2, 1987	)	Richard L. Raymond
	:	
For: DIBENZ[b, e]OXEPIN DERIVATIVE	)	

DECLARATION

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

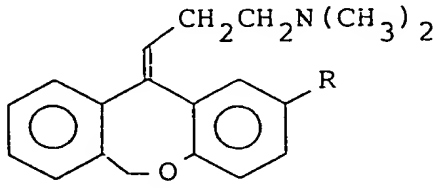
I, KENJI OHMORI, of 2-14-3, Fuyodai, Mishima-shi, Shizuoka-ken, Japan, hereby declare as follows:

I graduated from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University in March, 1970. I have been employed since April, 1970 by Kyowa Hakko Kogyo Co., Ltd. where I am engaged in the research and development of antiallergic agents. I studied the evaluation of antiallergic agents at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University from April, 1975 to March, 1976 at the direction of Kyowa Hakko Kogyo.

I am one of the inventors of the above-identified application and well acquainted with the prosecution thereof.

I have conducted the following experiments comparing, with respect to antiallergic activity (Anti PCA activity), anti-asthmatic activity and side effect (enhancement of hypnotic effect), between the compound of the present invention and the compounds disclosed in U.S. Patent

No. 4,871,865 as illustrated below.



Compound A : R=CH_2COOH
" B : R=COOH
" C : R=CH=CHCOOH

Compound A is cis-form of Compound No. 20 of the present invention. Compound B is disclosed in Example 1 (Compound 1) and Compound C is disclosed in Example 5 (Compound 10; see claim 3) in '865.

Experiment

1. Test for antiallergic activity

The experiment was conducted according to the same method as described at page 37, line 9 to page 38, line 23 of this application.

Effective dose producing the inhibition rate of 50% (ED_{50}) was determined by the Profit method. The results are shown in Table I.

2. Test for anti-asthmatic activity

Anti-asthmatic activity was evaluated by anaphylactic bronchial contraction method using guinea pigs. Hartley Male guinea pigs weighing 300 - 350g were used as test animals.

(A) Preparation of anti-EWA guinea pig serum

Anti-egg white albumin (EWA) serum of guinea pig was prepared according to the method of Santives et al. [J. Allergy clin. Immunol., 75, 582 (1976)]. Twenty mg of EWA

was dissolved in 10 mℓ of saline, and the solution was mixed with an equal volume of Freund's complete adjuvant, and emulsified. One mℓ of the emulsion was injected subcutaneously into foot pads of guinea pigs, and animals were actively sensitized. Blood was collected 4 weeks after the injection. Serum was separated and stored at -80°C.

(B) Effect on anaphylactic bronchoconstriction in passively sensitized guinea pigs.

Guinea pigs, 6 - 8 animals in each group, were passively sensitized with 1 mℓ of anti-EWA guinea pig serum. Twenty four hours after the animals were anesthesized, trachea and jugular vein were cannulated, and catheters were fixed. An artificial breathing apparatus (made by Takashima Instruments, Co.) and a bronchus path transducer (made by Ugo Basile, Co.) were connected. Self breathing was terminated by intravenous injection of gallamine (10 mg/kg), and anaphylactic bronchoconstriction was induced by intravenous injection of EWA (10 mg/kg). Air overflow volume from the side-arm of a tracheal cannula was recorded on polygraph (made by Nihon Photo Electronics, Co.) attached to the transducer. When the recording was finished, the bronchia was blocked by camp stoves and the bronchoconstriction at this time was expressed as a maximum (100%). The contraction at the following times was measured and expressed in %.

Test chemicals and saline (control) were given orally 1 hr before the administration of EWA. Broncho-constriction rates were measured 5 min after the administration of EWA using control or test chemical-treated animals, and suppression rates were determined. ED_{50}, 50% suppression dose, was calculated from the regression lines. The results are shown in Table I.

3. Enhancement of hypnotic effect

Male mice dd strain with body weight of 19 - 21g were used (4 - 5 mice in each group). Test chemicals or

saline (control) were administered orally, and sodium pentobarbital (15 mg/kg, unanesthetic dose) was injected 1 hr later. Disappearance of body-righting reflex was examined during 1 hr after the injection. The denominators indicate the number of animals used, and the numerators indicate the number of animals which showed disappearance of body-righting reflex under the above experimental conditions.

The results are shown in Table I.

Table I

Test compound	A	B	C
Antiallergic activity ED_{50} (mg/kg)	0.11	0.25	1.03
Anti-asthmatic activity ED_{50} (mg/kg)	0.011	0.053	0.080
Enhancement of hypnotic effect (dose: 200 mg/kg)	0/4	2/5	NT

NT: Not tested

4. Conclusion

As seen from the result of Table I, it is concluded that Compound A has more excellent antiallergic and anti-asthmatic activity and much less side effect (hypnotic effect) compared with Compound B. Compound A has more excellent anti-allergic and anti-asthmatic activity compared with Compound C.

The undersigned declarant declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 18th day of May, 1990

Kenji Ohmori

KENJI OHMORI